Entamoeba histolytica Schaudinn

50412™

Description

Strain designation: SAW 891 R Clone B
Deposited As: *Entamoeba histolytica* Schaudinn
Type strain: No

Storage Conditions

Product format: Frozen
Storage conditions: -80°C or colder for 1 week, vapor phase of liquid nitrogen for long-term storage

Intended Use

This product is intended for laboratory research use only. It is not intended for any animal or human therapeu
human or animal consumption, or any diagnostic use.

BSL 2

ATCC determines the biosafety level of a material based on our risk assessment as guided by the current editi
Microbiological and Biomedical Laboratories (BMBL), U.S. Department of Health and Human Services. It is your res
understand the hazards associated with the material per your organization's policies and procedures as well a
applicable regulations as enforced by your local or national agencies.

ATCC highly recommends that appropriate personal protective equipment is always used when handling vials.
that require storage in liquid nitrogen, it is important to note that some vials may leak when submersed in liq
will slowly fill with liquid nitrogen. Upon thawing, the conversion of the liquid nitrogen back to its gas phase r

vial exploding or blowing off its cap with dangerous force creating flying debris. Unless necessary, A
these cultures be stored in the vapor phase of liquid nitrogen rather than submersed in liquid nitroge

Certificate of Analysis

For batch-specific test results, refer to the applicable certificate of analysis that can be found at www.atcc.or

Growth Conditions

Medium:
ATCC Medium 2154: LYI Entamoeba medium
Instructions for complete medium:

ATCC Medium PRA-2154
(Quality controlled freeze-dried lots of this medium are commercially available from ATCC).

Temperature: 35°C
Atmosphere: Anaerobic
Culture system: Axenic

Handling Procedures

Storage and Culture Initiation

Frozen ampules packed in dry ice should either be thawed immediately or stored in liquid nitrogen. If liquid ni
facilities are not available, frozen ampules may be stored at or below -70°C for approximately one week. **Do n**
circumstance store frozen ampules at refrigerator freezer temperatures (generally -20°C). Storage of froze
this temperature will result in the death of the culture.

1. To thaw a frozen ampule, place in a 35°C water bath, until thawed (2-3 min). Immerse the ampule just s
   cover the frozen material. Do not agitate the ampule.

2. Immediately after thawing, aseptically transfer contents to a glass screw-capped tube conta
   2154. Screw cap on tightly and incubate on a 15° horizontal slant at 35°C.

Culture maintenance:

1. Ice culture at or near peak density for 10 min.
2. Gently invert culture 20 times.
3. Aseptically transfer a 0.1 and 0.25 ml aliquot to freshly prepared (no older than 7-10d) tubes of ATCC m
4. Screw caps on tightly and incubate at a 15° horizontal slant at 35°C.
5. Subculture every 10-14 days.

Reagents for cryopreservation: CPMB-5 Cryoprotective Solution

DMSO	1.0 ml
2.5 M Sucrose	0.8 ml
L-Cysteine/Ascorbic Acid Solution	0.2 ml
CPMB-2 Basal Solution	6.0 ml
HIBS	2.0 ml

CPMB-2 Basal Solution

Yeast Extract	60.0 g
K_2HPO_4	1.0 g
KH_2PO_4	0.6 g
NaCl	2.0 g
Distilled water	1.0 L

Autoclave for 15 minutes.

L-Cysteine/Ascorbic Acid Solution

L-Cysteine-HCL	1.0 g
Acorbic Acid	0.1 g
Distilled water	10.0 ml

Add 9.0 ml of distilled water to a 20 ml beaker and dissolve the first two components. While stirring, adjust th
10N NaOH (approximately 0.7 ml). Adjust final volume to 10 ml with distilled water and filter sterilize. Solution
soon after preparation. Discard any unused solution.

Cryopreservation:

1. Harvest cells from several cultures that are in the late logarithmic to early stationary phase of growth.
   vessels on ice for 10 min.

2. Invert tubes 20 times and centrifuge at 200 x g for 5 min.
3. While cells are centrifuging, prepare the cryoprotective solution.

   a) Place 1.0 ml of DMSO in a 16 x 125 mm screw-capped test tube and ice until solidified.
   b) Add 0.8 ml of the 2.5 M Sucrose solution, remove from ice and invert until the DMSO is liquefied. Re
   c) Add 0.2 ml of the L-Cysteine/Ascorbic Acid Solution to the DMSO solution and mix.
   d) Add 6.0 ml of the CPMB-2 Basal Solution and mix. e) Add 2.0 ml HIBS and mix.
4. Resuspend the cell pellets and pool to a final volume of approximately 10 ml with the supernatant. Mak determination of the cell density and adjust the concentration of the cells between 5 x 10^5/ml - 1 x 10^6 medium. If the cell concentration is below 5 x 10^5/ml, centrifuge the cell suspension and resuspend the volume that will yield the desired concentration.
5. After the cell concentration is adjusted, centrifuge as in step 2.
6. Remove as much supernatant as possible and determine the volume removed.
7. Resuspend the cell pellet with a volume of the cryoprotective solution equal to the volume of the supe removed. Invert the tube several times to obtain a uniform cell density.
8. Dispense 0.5 ml aliquots into 1.0 - 2.0 ml plastic sterile cryules (special plastic vials for cryopreservation)
9. Place the vials in a controlled rate freezing unit. Use the following cooling cycle: From room temperatu 10°C/min to the heat of fusion; from the heat of fusion to -40°C, cool at -1°C/min. At -40°C plunge into The cooling cycle should be initiated no less than 15 and no more than 30 minutes after the addition of cell preparation.
10. Store ampules in a liquid nitrogen refrigerator until needed.
11. To establish a culture from the frozen state, place an ampule in a 35°C water bath, until thawed (2-3 mi vial just sufficient to cover the frozen material. Do not agitate the ampule.
12. Transfer contents of thawed ampule to a 16 x 125 mm screw-capped borosilicate glass test tube contai ATCC medium 2154.
13. Screw cap on tightly and incubate at a 15° horizontal slant at 35°C. Observe the culture daily and transf trophozoites are observed.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: *Entar* Schaudinn (ATCC 50412)

References

References and other information relating to this material are available at www.atcc.org.

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Revision

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